REMARKS

Reconsideration of the above-identified application in view of the remarks below is respectfully requested.

No claims have been canceled, amended or added in this paper. Therefore, claims 1-30 are pending. Of these claims, claims 17-20 and 23-30 have been withdrawn as being drawn to a non-elected invention or a non-elected species. Therefore, claims 1-16 and 21-22 are under active consideration.

Claims 1-13 stand rejected under 35 U.S.C. 102(a) "as being anticipated by Bransteitter et al. (PNAS USA, vol. 100, pp. 4102-4107, April 2003; cited in the IDS)." In support of the rejection, the Patent Office states the following:

> Regarding claim 1, Bransteitter et al. teach a method for the detection of cytosine methylations in DNA (Abstract) comprising the steps of:
> a) bringing the DNA to be investigated into contact with a cytidine deaminase, whereby the cytidine deaminase deaminates cytidine and 5-methylcytidine at different rates (page 4102, paragraphs 3-5),
> b) investigating the partially deaminated DNA with respect to its sequence (page 4102, last paragraph; page 4103, first and second paragraph), and
> c) concluding from the presence or the proportion of deaminated postions the methylation status of the DNA to be investigated in said positions (Fig. 1; Fig. 2).
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> Regarding claim 2, Bransteitter et al. teach AID (page 4102, fourth paragraph).
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> Regarding claims 3 and 4, Bransteitter et al. teach single-stranded and partially-single stranded DNA (page 4102, third paragraph; Table 1).
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> Regarding claims 5-7, Bransteitter et al. teach single stranded regions being between 3 and 20 nucleotides long, between 5 and 12 nucleotides long and 9 nucleotides long (Table 1, page 4106).

> Regarding claims 8 and 9, Bransteitter et al. teach oligomers between the length of 20 to 150 nucleotides and 35-60 nucleotides (Table 1, page 4106).
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> Regarding claims 10 and 11, Bransteitter et al. teach oligomers concentration of 100 nM (page 4102, fifth paragraph), anticipating the claimed ranges.
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> Regarding claims 12 and 13, Bransteitter et al. teach amplification of the deaminated fragment using a polymerase (page 4103, second paragraph).

Elsewhere in the Office Action, the Patent Office states the following:

> Applicant's arguments filed November 6, 2009 have been fully considered but they are not persuasive.
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> Regarding the rejection of claims 1-13 under 35 U.S.C. 102(b) as anticipated by Bransteitter et al., Applicants argue that Bransteitter et al. do not teach the step of c) of claim 1, i.e., "concluding, from the presence or the proportion of deaminated positions, the methylation status of the DNA to be investigated in said positions".
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> However, it is very clear from the legend to Fig. 1 that this is what Bransteitter et al. do. The legend to Fig. 1 (a) states: "Assay 1 detects dC deamination by using UDG and APE." Therefore, the fact that there is a result indicating deaminated cytosine is a conclusion that the methyl group was present on that cytosine. Therefore this limitation is anticipated.

Applicant respectfully traverses the subject rejection.

At the outset, Applicant respectfully submits that there may be some confusion on the part of the Patent Office as to what the teachings of Bransteitter et al. are and how those teachings relate to the present invention. Therefore, in an effort to provide clarity, Applicant is presenting the comments below.

The present invention is directed at a method for detecting the methylation status of cytosine bases located within a length of DNA. The methylation status of such cytosine bases is either methylated (in which case the base is "5-methylcytosine") or unmethylated (in which case the base is simply "cytosine"). As noted in the present specification, for example, in the paragraph bridging pages 1 and 2, the methylation of cytosine plays an important biological role and is involved, among other things, in the regulation of transcription, in genetic imprinting, and in tumorigenesis. Unfortunately, the determination of whether a cytosine base is methylated or unmethylated is a difficult one because cytosine and 5-methylcytosine have the same base-pairing behavior. As a result, conventional detection methods based on hybridization cannot be used to distinguish cytosine and 5-methylcytosine. Moreover, the information as to whether a cytosine base is methylated or not is completely lost in a PCR amplification.

The present invention addresses the above difficulty, in part, by contacting the DNA under investigation with cytidine deaminase. Cytidine deaminase converts cytidine (cytidine being the nucleotide that comprises cytosine) into uridine. Moreover, cytidine deaminase converts cytidine and 5-methylcytidine at different rates. As a result, cytidine deaminase can convert unmethylated cytosine into uracil whereas methylated cytosine remains essentially unchanged. Therefore, once the DNA under investigation has been brought into contact with the cytidine deaminase, the partially deaminated DNA may be investigated with respect to its sequence, and one may then conclude, from the presence or the proportion of deaminated positions, the methylation status of the DNA in those positions.

Bransteitter et al. is primarily directed at investigating the activity of AID (activation-induced cytidine deaminase) in deaminating deoxycytidine found in different forms of DNA and RNA, as opposed to free deoxycytidine (dC) in solution. In particular, Bransteitter et al. discloses that "AID catalyzes deamination of dC residues on single-stranded DNA *in vitro* but not on double-stranded DNA, RNA-DNA hybrids, or RNA" and "has no measurable deaminase activity on single-stranded DNA unless pretreated with RNase to remove inhibitory RNA bound to DNA." Bransteitter et al. also discloses that "AID catalyzes dC → dU [deoxyuridine] deamination activity most avidly on double-stranded DNA substrates containing a small "transcription-like" single-stranded DNA bubble."

In the Amendment filed November 4, 2009, Applicant noted that Bransteitter et al. does not teach or suggest, amongst other things, step (c) of claim 1, namely, concluding, from the presence or the proportion of deaminated positions, the methylation status of the DNA to be investigated in said positions. In particular, Applicant noted that the portions of Bransteitter et al. apparently being relied upon by the Patent Office, namely, Figs. 1 and 2 are completely silent about the **methylation** status of the DNA under investigation.

Notwithstanding the above, the Patent Office is stating in the outstanding Office Action that the legend to Fig. 1, in particular, the statement that "Assay 1 detects dC deamination by using UDG and APE," demonstrates that Bransteitter et al. discloses step (c) of claim 1. In fact, the Patent Office continues by stating that "the fact that there is a result indicating deaminated cytosine is a conclusion that the methyl group was present on that cytosine."

Applicant respectfully disagrees with the Patent Office's line of reasoning. **Bransteitter et al. does not teach or suggest treating DNA with AID to distinguish cytosine and 5-methylcytosine.** In fact, the only comment in Bransteitter et al. that is even remotely related is an isolated comment in the paragraph bridging the left and right columns on page 4106 ("AID exhibits ≈ 10-fold higher specific activity on ssDNA for the deamination of dC → dU compared with 5-methylcytosine → T (Fig. 4b)." However, merely stating the differential activity of AID towards dC and 5-methylcytosine does not teach or suggest using AID to distinguish cytosine and 5-methylcytosine. **Moreover, nothing in Figs. 1 and 2 of Bransteitter et al. has anything to do with the differential activity of AID with respect to cytosine and 5-methylcytosine nor does anything anywhere in Bransteitter et al. teach or suggest using this property to determine the methylation status of cytosine bases in DNA. In fact, as noted above, Figs. 1 and 2 are completely silent about whether the DNA under investigation includes any methylated cytosines.** To the extent that the Patent Office appears to be arguing that Bransteitter et al. detects dC deamination and that this fact necessarily results in a conclusion that a methyl group was present on the cytosine, Applicant notes that **Bransteitter et al. does not teach or suggest using dC deamination to detect cytosine methylation.** (Moreover, although not taught or suggested in Bransteitter et al., Applicant wishes to point out that dC deamination is more likely to suggest an unmethylated cytosine, as opposed to a methylated cytosine.)

In short, Bransteitter et al. does not recognize the methylation status of the DNA under investigation in its Figs. 1 and 2 nor does it teach or suggest that the differential activity of AID can

be used to distinguish methylated and unmethylated cytosines. Consequently, it cannot be said that Bransteitter et al. teaches or suggests the concluding step of claim 1.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

Claims 12-16, 21 and 22 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over Bransteitter et al. (PNAS USA, vol. 100, pp. 4102-4107, April 2003; cited in the IDS) and Olek et al. (U.S. Patent No. 7,229,759 B2)." In support of the rejection, the Patent Office states the following:

> A) Bransteitter et al. teach detection of the converted uracil residues using primer extension and ddA, but do not teach PCR or real-time PCR or using blocker oligonucleotides in the amplification reaction.
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> B) Regarding claims 12-14, 21 and 22, Olek et al. teach detection of deaminated cytosines resulting from bisulfite reaction using real-time PCR (col. 5, lines 37-53; col. 13, lines 45-59).
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> Regarding claim 15, Olek et al. teach methylation-specific primers (col. 2, lines 56-67; col. 3, lines 1, 2; col. 11, lines 15-31).
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> Regarding claim 16, Olek et al. teach using blocking oligonucleotides during amplification (col. 6, lines 3-20 and 38-67; col. 11, lines 29-49).
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> It would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to have used the amplification methods of Olek et al. with blocking oligonucleotides to detect the converted cytidines in the method of Bransteitter et al. The motivation to do so is provided by Olek et al. (col. 13, lines 52-59 and col. 11, lines 67 and col. 12, lines 1-3):
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> "A particularly preferred variant of the method, however, is the simultaneous detection of qualifier positions and classifier positions in one experiment, which can be achieved by the use of TaqMan or LightCycler technology variants. Additionally fluorescently labeled oligonucleotides are to be added to the oligonucleotides, which provide for a preferred amplification of the DNA to be investigated, and the change in fluorescence is measured during the PCR reaction. In principle, since the DNA to be

investigated is amplified, information on the methylation status of different classifier CpG positions is obtained predominantly also directly from this change in fluorescence. Since different oligonucleotides are each preferably provided with different fluorescent dyes, a distinction of the change in fluorescence during the PCR is also possible, separately for different positions."

"If only one small group of CpGs is available and still a high amount of background DNA has to be blocked, it is therefore preferred that one part of this group of CpGs is covered by a methylation specific primer and the other part is covered by a methylation specific blocking probe, and the binding site of this non-extendible probe could ideally even overlap with the binding site of the primer. This way, high relative sensitivity, this means highly preferred amplification of the DNA to be analyzed while suppressing the background DNA, can be achieved with only a small group of CpGs as Qualifier positions."

Elsewhere in the Office Action, the Patent Office states the following:

Regarding the rejection of claims 12-16, 21 and 22 under 35 U.S.C. 103(a) over Bransteitter et al. and Olek et al., Applicants argue that:

"Because Bransteitter explicitly states that deamination efficiencies were calculated from extension reactions with ddA mix or from extension reactions with ddG mix, a person of ordinary skill in the art would have considered the use of such ddA mix or ddG mix as necessary for detecting deamination of cytosines, i.e., converted uracils.

For this reason, even if a person of ordinary skill in the art would have had the idea of combining the method of Bransteitter with the method of Olek, he would have ended up with a method wherein said ddA or said ddG mixes are used for primer extension, irrespective of whether this primer extension is a single extension or part of a PCR, real-time PCR or conducted in the presence of blocking oligonucleotides. However, according to the claimed method, a primer extension is not necessary. According to step (b) of claim 1, the sequence of partially deaminated DNA is analyzed. According to claim 12 (as well as claims 13-16 dependent therefrom), such analysis may comprise amplification, preferably a polymerase mediated amplification, more preferably by means of PCR, and most

> preferably either in the presence of methylation-specific primers or a methylation specific blocker oligonucleotide. In contrast to the teaching of Bransteitter and thus in contrast to a combination of Bransteitter and Olek, the use of a mixture of three dNTPs with either ddA or ddG is not necessary."
>
> One of ordinary skill in the art would have clearly understood that the amplification method of Olek et al. is not to be combined with the elongation method of Bransteitter et al., but rather that it is a substitute for it.

Applicant respectfully traverses the subject rejection. Claims 12-16, 21 and 22 depend directly or indirectly from claim 1. Claim 1 is patentable over Bransteitter et al. for at least the reasons given above. Olek et al. fails to cure all of the deficiencies of Bransteitter et al. with respect to claim 1. Therefore, based at least on their respective dependencies from claim 1 and based on the reasons already of record, claims 12-16, 21 and 22 are patentable over the applied combination of Bransteitter et al. and Olek et al.

Accordingly, for at least the above reasons, the subject rejection should be withdrawn.

In conclusion, it is respectfully submitted that the present application is now in condition for allowance. Prompt and favorable action is earnestly solicited.

If there are any fees due in connection with the filing of this paper that are not accounted for, the Examiner is authorized to charge the fees to our Deposit Account No. 11-1755. If a fee is

required for an extension of time under 37 C.F.R. 1.136 that is not accounted for already, such an extension of time is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

Kriegsman & Kriegsman

By: _____

Daniel S. Kriegsman
Reg. No. 40,057
30 Turnpike Road, Suite 9
Southborough, MA 01772
(508) 481-3500

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on 7-15-10.

Daniel S. Kriegsman
Reg. No. 40,057
Dated: 7-15-10